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Prevalence and genotypes of *Giardia duodenalis* in 1–2 year old dairy cattle

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Abstract

To determine the prevalence of *Giardia* genotypes in 12–24 month old dairy heifers, fecal specimens were collected from two farms each in Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida. Specimens, cleaned of fecal debris and concentrated using CsCl density gradient centrifugation, were subjected to PCR and DNA sequence analysis. Prevalence of *Giardia* infection, ranged from 11% to 75% on 14 farms with an average prevalence of 36% (204 positive cattle out of 571 examined). DNA sequence analysis of the 16S rRNA gene revealed 91% of the 204 *Giardia* isolates were Assemblage E, and 9% were Assemblage A. The prevalence of these genotypes varied greatly from farm to farm, with four farms having exclusively Assemblage E *Giardia*. Overall, Assemblage E was present in 33% of all animals tested and Assemblage A was present in 3% of the animals. Thus, while many of the heifers were infected with a genotype that is not known to be infectious for humans, 1–2 year old heifers on 10 of 14 farms did harbor zoonotic Assemblage A *Giardia*. Therefore, heifers cannot be overlooked as a potential source of human infectious cysts in the environment, with some farms representing a much higher risk than others.

Keywords: Calves; Genotype; *Giardia* spp.; PCR; Prevalence

1. Introduction

Giardia duodenalis (syn. *Giardia lamblia*, *Giardia intestinalis*) is a commonly identified intestinal parasite of mammals, including humans. Giardiasis has been the most commonly diagnosed disease associated with drinking water in United States (Lee et al., 2002), however, the potential sources of cysts infectious for humans are not well known. Identification of animal sources of *Giardia* is complicated by the fact that although genetic and biological differences exist within the *G. duodenalis* complex, subtypes are morphologically indistinguishable (Thompson et al., 2000). Thus, it appears that organisms classified under the umbrella of

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G. duodenalis have different biological potentials for infecting humans or other animal species. Molecular analysis has proven useful for identifying genotypes or Assemblages of *G. duodenalis* and some of their associated hosts (Monis et al., 1999, 2003). Assemblages A and B appear to have the widest host ranges, including humans, cattle, other domesticated animals and wild animal species; Assemblages C and D infect dogs, Assemblage E infects hoofed livestock, Assemblage F infects cats, and Assemblage G infects rats (Monis et al., 2003).

The prevalence of *Giardia* infection in cattle varies markedly. While many point prevalence studies of cattle report a significant percentage of *Giardia*-infected animals, cumulative prevalence often reaches 100% (Xiao and Herd, 1994; Olson et al., 1997a,b; Ruest et al., 1998; O'Handley et al., 1999; Ralston et al., 2003). Despite the abundant prevalence information based on microscopy, there is little information on the genotypes that are present in infected cattle in the United States. Assemblage E was the predominant genotype found in Canadian dairy and beef cattle and Australian dairy cattle (O'Handley et al., 2000; Appelbee et al., 2003), but a small percentage of Assemblage A has been found in Canada, Australian and the Netherlands (O'Handley et al., 2000; Huetink et al., 2001; Appelbee et al., 2003). Analysis of three bovine specimens from New York indicated the presence of Assemblage A in cattle as well (van Keulen et al., 2002).

In two multi-state prevalence studies for *Giardia* in the eastern United States, pre-weaned calves (<2 months of age) and post-weaned calves (2–12 months of age) were found infected with both Assemblages E and A. On average, Assemblage E was found in 85 and 87% of pre- and post-weaned calves, respectively (Trout et al., 2004, 2005). Assemblage A was found in 15 and 13% of pre- and post-weaned calves, respectively (Trout et al., 2004, 2005). Although there was significant farm-to-farm variation, Assemblage E was found in pre- and post-weaned animals on all farms. Assemblage A, however, was not detected on all farms. In pre-weaned calves, the percentage of isolates that were Assemblage A ranged from a low of 0% of isolates on 7 of 14 farms to a high of 45% of isolates on a farm in New York (Trout et al., 2004). Likewise in post-weaned calves, Assemblage A ranged from 0% of the isolates on 5 of 14 farms to 67% of isolates on a Maryland farm (Trout et al., 2005). Thus, depending on the farm, a significant percentage of the *Giardia* isolated from pre- and post-weaned calves had zoonotic potential. These previous studies on the prevalence of *G. duodenalis* genotypes in US dairy cattle were geographically widespread and involved 407 and 456 calves in the pre- and post-weaned groups, respectively. Similar data, however, are not available for older dairy animals. Therefore, the present study was undertaken to assess the prevalence of *G. duodenalis* genotypes in 1–2 year dairy heifers over a multi-state area.

2. Materials and methods

2.1. Dairy farms

Two commercial dairy farms in Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida were selected based on an expected availability of 30 heifers in the appropriate age range for sampling. Eleven of the 14 farms that participated in a survey of post-weaned calves (Trout et al., 2005) also participated in the current study. Three farms VA-1, NC-1 and NC-2 were unable to participate and were replaced with different farms (VA-3, NC-3 and NC-4, respectively). PA-2 was replaced with PA-3 during the previous study.

2.2. Animals and housing

Dairy heifers between the ages of 12 and 24 months of age were randomly selected for sampling on each farm. The number of animals sampled and their ages were dependent on the population on a given farm. The number of useful specimens obtained ranged from 29 to 62 per farm (Table 1). Heifers were generally housed in groups, in large pens, either completely or partially covered by a roof. The exceptions were MD-2, VA-3, FL-1, and FL-2, where animals grazed on pasture, and VA-2, where animals were housed in a large free-stall barn.

2.3. Fecal sample collection and processing

Fecal samples were collected and processed as described by Trout et al. (2004). Briefly, feces were

Table 1
Farm designations, number of calves sampled on each farm, and prevalence of *Giardia duodenalis* and genotypes in 12–24 month old dairy heifers on 14 farms in seven East Coast states

State	Farm	Number of animals sampled	Number positive by PCR	Assemblage (percent of PCR positive isolates)	
				A	E
Vermont	VT-1	31	17 (55%)	29	71
	VT-2	39	26 (67%)	4	96
New York	NY-1	34	18 (53%)	0	100
	NY-2	37	4 (11%)	25	75
Pennsylvania	PA-1	62	32 (52%)	3	97
	PA-3	53	24 (45%)	4	96
Maryland	MD-1	50	6 (12%)	33	67
	MD-2	43	11 (26%)	9	91
Virginia	VA-2	42	6 (14%)	0	100
	VA-3	29	6 (21%)	0	100
North Carolina	NC-3	36	27 (75%)	7	93
	NC-4	29	6 (21%)	50	50
Florida	FL-1	47	12 (26%)	0	100
	FL-2	39	9 (23%)	11	89
Total		571	204 (36%)	9	91

collected from each calf into plastic screw cap specimen cups and processed within 4 days of collection. Fifteen grams of feces were mixed with water, passed through a 45 μm screen. The samples were then subjected to density gradient centrifugation using CsCl (1.4 g/ml). Following centrifugation, the top 4 ml of supernatant were aspirated from each sample and transferred into a 15 ml tube, and samples were washed twice with dH_2O; the final pellet was suspended in 500 μl of dH_2O.

2.4. DNA extraction

Total DNA was extracted from each CsCl-cleaned fecal sample using a DNeasyTissue Kit (Qiagen, Valencia, California) with a slightly modified protocol. The protocol, described below, utilized reagents provided by the manufacturer. A total of 50 μl of processed feces were suspended in 180 μl of ATL buffer and thoroughly mixed by vortexing. To this suspension, 20 μl of Proteinase K (20 mg/ml) was added, and the sample was thoroughly mixed. Following an overnight incubation of the mixture at 55 °C, 200 μl of AL buffer was added. The remaining protocol followed manufacturer's instructions with one exception. To increase the quantity of recovered DNA, the nucleic acid was eluted in 100 μl of AE buffer.

2.5. Polymerase chain reaction and DNA sequence analysis

A fragment of the ssu-rRNA (~292 bp) gene was amplified by PCR as previously described (Hopkins et al., 1997). PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining.

PCR products were purified using EXO-SAP enzyme (USB Corporation, Cleveland, Ohio). Purified products were sequenced with the same PCR primers used for the original amplification in 10 μl reactions, Big Dye™ chemistries, and an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, California). Each sample was sequenced in both directions. Sequence chromatograms from each strand were aligned and inspected using Lasergene software (DNASTAR Inc., Madison, Wisconsin). All of the PCR positive samples were sequenced.

3. Results

The number and location of calves infected with *Giardia* determined PCR are shown in Table 1. Of 571 calves on 14 farms examined by PCR, 204 (36%) were *Giardia* positive. The prevalence of *Giardia* infection varied considerably across farms, with the lowest prevalence (11%) on NY-2 and the highest prevalence (75%) on NC-3. Overall, on 5 of the 14 farms greater than 50% of the calves were found to be positive for *Giardia*.

The percentages of *G. duodenalis* genotypes found on each farm are presented in Table 1. Two genotypes were identified: Assemblage E, which has been reported to infect only hoofed-livestock, and Assemblage A, which is infectious for humans and a number of other mammals. Across all farms, 9% of the *Giardia* positive animals were infected with Assemblage A, and 91% were infected with Assemblage E. Assemblage A was not found on all farms. On farms where this Assemblage was present, the lowest percentage was on PA-1 (3%) and the highest percentage was on NC-4 (50%).

4. Discussion

G. duodenalis infections were detected in 12–24 month old dairy heifers on all 14 farms examined, with prevalence by PCR ranging from 11% to 75%. Previous point prevalence studies have also reported a wide range in the number of *Giardia* infected animals (Xiao, 1994; Xiao and Herd, 1994; Olson et al., 1997a,b; O'Handley et al., 1999, 2000; Trout et al., 2004, 2005). Additionally, point prevalence studies are likely to underestimate the true number of infected animals at any given time especially near the end of the infection when cyst excretion can be intermittent (Buret et al., 1990). In fact, cumulative prevalence studies for a given farm often report 100% of the animals infected. (Xiao and Herd, 1994; O'Handley et al., 1999). Because the data presented herein were collected in a point prevalence study, it is likely that actual prevalence in these 12–24 month old heifers is underestimated as well.

The prevalence of *Giardia* genotypes was determined by DNA sequence analysis of the 16S rRNA gene for every PCR positive sample. Thus, sequence data were obtained for 204 cattle samples. Assemblage A *Giardia* (commonly seen in human *Giardia* infections) was detected at varying levels in calves on 10 of the 14 farms. Assemblage E *Giardia*, with a host range limited to hoofed-livestock, was detected in calves on all farms, with four farms having exclusively Assemblage E. On farms where Assemblage A was detected, this genotype represented between 3% (PA-1) and 50% (NC-4) of the isolates. Thus, while Assemblage A represented 9% of the isolates in the study as a whole, on four farms, this genotype represented greater that 20% of the isolates. Similar variation among farms was seen in two previous studies conducted by our laboratory. In the first study (pre-weaned calves), Assemblage A *Giardia* was detected on 7 of 14 farms, and represented 15% of the total *Giardia*, but exceeded 20% of the isolates on six farms (Trout et al., 2004). In the second study (post-weaned calves), Assemblage A was detected on 9 of 14 farms and represented 13% of the total, but exceeded 20% of the isolates on four farms. Thus averaging data over this wide geographical area appears to obscure the risk that might be posed by a particular farm. The reason for such wide variation in the levels of Assemblage A between farms is unclear.

In looking at the prevalence of Assemblages A and E across different age groups, Assemblage A was detected in 0–10% of the animals between 1 week and 21 months of age, whereas Assemblage E was detected in 5–67% of the same animals (Trout et al., 2004, 2005, and current study). Similar studies of *Cryptosporidium* species and genotypes (Santín et al., 2004; Fayer et al., 2006), reported the pre-weaned calves were the primary source of the zoonotic species, *Cryptosporidium parvum*, while post-weaned calves and 1–2 year old heifers were primarily infected with species and genotypes that were not infectious for humans. This situation does not appear to exist for *Giardia*, in that the percentage of Assemblage A varied little with age, indicating that dairy heifers through atleast 21 months of age can still produce cysts that are potentially infectious to humans. It is not clear from the current study if the lack of detectable Assemblage A in animals over 21 months of age defines an upper age limit for this genotype, however, an ongoing study to examine dairy cows >2 years of age should answer this question. Additionally, data from our previous studies (Trout et al., 2004, 2005) indicates that Assemblage A can be present on a particular farm 1 year and not the next or conversely, not present 1 year and then present the next. However, such a comparison is confounded by both sampling year and the age group sampled.

We have also not been able to determine if animals can harbor both genotypes simultaneously. Within a given sample, our analysis methods would tend to obscure the genotype present in low numbers in favor of the genotype present in greater numbers. It is also unclear if infection with one genotype confers resistance or immunity to the other genotype, and ongoing longitudinal study of heifers on the same farm will hopefully answer this question. While it is tempting to focus on Assemblage A because of the potential risk of human infection, it should be noted that on average 36% of these 1–2 year old dairy heifers are infected with some genotype of *G. duodenalis*. It is not known if *Giardia* infection causes any clinical problems or loss of production capacity in these animals.

The present study demonstrates substantial levels of *Giardia* infection in older dairy heifers and provides genotype data for all *Giardia* positive samples on a small number of farms over a wide geographical area. The presence of Assemblage A on 10 of 14 of the farms and its high prevalence on several farms indicate that 1–2 year old dairy heifers should be considered a potential source of zoonotic *G. duodenalis* cysts and that the distribution and prevalence of this genotype can be unpredictable.

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